Cell-Lysate Experiment of CCH2:

Materials Needed:
250 mL Erlenmeyer flasks
M9 minimal salt media
Chloramphenicol (25mg/ml)
1M IPTG
200 mM 6-CNA dissolved in methanol
250 mL Erlenmeyer flasks with 50 mL LB
Spectrophotometer
Cuvette
20mM (+80mM NaCl) MOPS Buffer
7M Urea Buffer
Sonicator
1.5mL Eppendorf tubes
2 mL Eppendorf Tubes
10mL glass test tubes
Ethyl Acetate
12M Hydrochloric Acid
pH paper
GC vials
GC vial micro-inserts
GC vial caps
Nitrogen Gas
Pyridine
BSTFA + TMSC 99:1 (Derivatizing Agent)
Vortex
Centrifuge
GC/MS
Pipet

M9 minimal salt media components:
33.9g/L Na_2HPO_4
15g/L KH_2PO_4
5g/L NH_4Cl
2.5g/L NaCl

Protocol:
1) See Determination of CCH2 rate protocol and repeat steps 1-6 [1].
2) Four aliquots are taken of 1.5mL and transferred into four Eppendorf tubes.
3) The samples were centrifuged at 15000rpm for 30 minutes at 4°C and the supernatant is discarded.
4) The cell pellets are resuspended in 800 µL of MOPS.
5) Two of the four resuspended pellets were sonicated at 1W two times. The other two were sonicated on the lowest setting four times. The aliquots

were sonicated for ten seconds and had one minute of wait time before the subsequent sonication, while being kept on ice.

6) One of each sonication time samples is centrifuged at 15000rpm for 30 minutes at 4°C and the supernatant transferred to Eppendorf tubes.
7) The other sonication time samples stay on ice without being centrifuged.
8) 380 µL of each sample are transferred into Eppendorf tubes and 20 µL of 6-CNA was added.
9) The samples are incubated overnight at 30°C.
10) The supernatant fractions are extracted via the liquid-liquid extraction protocol, with modified quantities of 12M HCL and Ethyl Acetate [2].
11) The samples were then prepared via the GC-MS sample preparation protocol [3].

Other Protocols:

[1] Step 1-6 of protocol- Determination of CCH2 rate:
[2] Liquid-Liquid Extraction for isolation of 6-Chloronicitinoic Acid and 6-Hydroxynicitinoic Acid
[3] Gas Chromatography-Mass spectroscopy conditions to run 6-Chloronicitinoic Acid and 6-hydroxynicitinoic Acid and sample preparation: